

Listing of the Claims

This listing of claims will replace all prior versions, and listings of claims in the application.

1-21. (Canceled)

22. (Original) A method of cloning a nucleic acid molecule comprising:

- (a) providing a first nucleic acid segment flanked by a first and a second recombination site and a second nucleic acid segment flanked by a third and a fourth recombination site, wherein none of the recombination sites flanking the first and second nucleic acid segment is capable of recombining with any of the other sites flanking the first and second nucleic acid segment;
- (b) providing a vector comprising a fifth, sixth, seventh and eighth recombination site, wherein each of the fifth, sixth, seventh and eighth recombination sites is capable of recombining with one of the first, second, third or fourth recombination site; and
- (c) conducting a recombination reaction such that the two nucleic acid segments are recombined into the vector thereby cloning the first and the second nucleic acid segments.

23-34. (Canceled)

35. (Previously Presented) A method of cloning two or more nucleic acid segments, comprising:

- (a) providing two or more nucleic segments, each segment flanked by two recombination sites which do not recombine with each other;
- (b) providing a vector comprising a number of recombination sites equal to twice the number of nucleic acid segments, wherein each of the recombination sites is capable of recombining with one of the recombination sites flanking one of the nucleic acid segments; and

- (c) conducting a recombination reaction such that the nucleic acid segments are recombined into the vector thereby cloning the nucleic acid segments,

wherein transcription of at least two of the nucleic acid segments results in the production of a single RNA.

36. (Original) The method of claim 35, wherein at least one of the nucleic acid segments produces a sense RNA strand upon transcription and at least one of the nucleic acid segments produces an antisense RNA strand upon transcription.

37. (Original) The method of claim 36, wherein the sense RNA and antisense RNA have at least one complementary region and are capable of hybridizing to each other.

38. (Canceled)

39. (Canceled)

40. (Previously Presented) The method of claim 35, wherein the nucleic acid segments comprise one or more libraries comprise nucleic acid molecules which encode variable domains of antibody molecules.

41. (Original) The method of claim 40, wherein the one or more libraries comprise nucleic acid molecules which encode variable domains of antibody light and heavy chains.

42. (Previously Presented) The method of claim 35, further comprising screening to identify nucleic acid molecules which encode proteins having binding specificity for one or more antigens.

43-46. (Canceled)

47. (Original) A method of cloning at least one nucleic acid molecule comprising:

- (a) providing a first, a second and a third nucleic acid segment, wherein the first nucleic acid segment is flanked by a first and a second recombination site, the second nucleic acid segment is flanked by a third and a fourth recombination site and the third nucleic acid segment is flanked by a fifth and a sixth recombination site, wherein the second recombination site is capable of recombining with the third recombination site and none of the first, fourth, fifth or sixth recombination sites is capable of recombining with any of the first through sixth recombination sites;
- (b) providing a vector comprising a seventh and an eighth recombination site flanking a first negative selectable marker and comprising a ninth and a tenth recombination site flanking a second negative selectable marker, wherein none of the seventh through tenth recombination sites can recombine with any of the seventh through tenth recombination sites;
- (c) conducting a first recombination reaction such that the second and the third recombination sites recombine; and
- (d) conducting a second recombination reaction such that the first and the fourth recombination sites recombine with the seventh and the eighth recombination sites and the fifth and the sixth recombination sites recombine with the ninth and the tenth recombination sites thereby cloning the first, second and third nucleic acid segments.

48. (Original) A method of cloning at least one nucleic acid molecule comprising:

- (a) providing a first, a second and a third nucleic acid segment, wherein the first nucleic acid segment is flanked by a first and a second recombination site, the second nucleic acid segment is flanked by a third and a fourth recombination site and the third nucleic acid segment is flanked by a fifth and a sixth recombination site, wherein the second recombination site is capable of recombining with the

third recombination site and the fourth recombination site is capable of recombining with the fifth recombination site;

- (b) providing a vector comprising a seventh and an eighth recombination site; and
- (c) conducting at least one recombination reaction such that the second and the third recombination sites recombine and the fourth and the fifth recombination sites recombine and the first and the sixth recombination sites recombine with the seventh and the eighth recombination sites respectively, thereby cloning the first, second and third nucleic acid segments.

49. (Original) The method of claim 48, wherein the recombination reaction is conducted in the presence of one or more recombination proteins under conditions which favor the recombination.

50. (Original) A method of cloning n nucleic acid fragments, wherein n is an integer greater than 2, comprising:

- (a) providing a 1st through an n^{th} nucleic acid segment, each segment flanked by two recombination sites, wherein the recombination sites are selected such that one of the two recombination sites flanking the i^{th} segment, n_i , reacts with one of the recombination sites flanking the n_{i-1}^{th} segment and the other recombination site flanking the i^{th} segment reacts with one of the recombination sites flanking the n_{i+1}^{th} segment;
- (b) providing a vector comprising at least two recombination sites, wherein one of the two recombination sites on the vector reacts with one of the sites on the 1st nucleic acid segment and another site on the vector reacts with a recombination site on the n^{th} nucleic acid segment; and
- (c) conducting at least one recombination reaction such that all of the nucleic acid fragments are recombined into the vector.

51. (Original) The method of claim 50, wherein the recombination reaction is conducted in the presence of one or more recombination proteins under conditions which favor the recombination.

52. (Original) The method of claim 51, wherein the recombination proteins comprise one or more proteins selected from the group consisting of: (a) Cre; (b) Int; (c) IHF; (d) Xis; (e) Fis; (f) Hin; (g) Gin; (h) Cin; (i) Tn3 resolvase; (j) TndX; (k) XerC; and (l) XerD.

53. (Original) The method of claim 50, wherein the recombination sites of the nucleic acid segments and the vector comprise one or more recombination sites selected from the group consisting of: (a) *lox* sites; (b) *psi* sites; (c) *dif* sites; (d) *cer* sites; (e) *frt* sites; (f) *att* sites; and (g) mutants, variants, and derivatives of the recombination sites of (a), (b), (c), (d), (e), or (f) which retain the ability to undergo recombination.

54. (Original) The method of claim 53, wherein the recombination sites which recombine with each other comprise *att* sites having identical seven base pair overlap regions.

55-68. (Canceled)

69. (Previously Presented) A method of joining two or more segments of nucleic acid, comprising:

- (a) providing two or more segments of nucleic acid, each segment comprising at least one recombination site capable of recombining with a recombination site present on the other segment; and
- (b) contacting the segments with one or more recombination proteins under conditions causing recombination between the recombination sites, thereby joining the segments,

wherein the expression product is a ribozyme or an inhibitory RNA molecule.

70-76. (Canceled)

77. (Original) A method of joining n nucleic acid segments, wherein n is an integer greater than 2, comprising:

- (a) providing a 1st through an n^{th} nucleic acid segment, each segment flanked by two recombination sites, wherein the recombination sites are selected such that one of the two recombination sites flanking the i^{th} segment, n_i , reacts with one of the recombination sites flanking the n_{i-1} th segment and the other recombination site flanking the i^{th} segment reacts with one of the recombination sites flanking the n_{i+1} th segment; and
- (b) contacting the segments with one or more recombination proteins under conditions causing the segments to join.

78. (Original) The method of claim 77, wherein the recombination proteins comprise one or more proteins selected from the group consisting of: (a) Cre; (b) Int; (c) IHF; (d) Xis; (e) Fis; (f) Hin; (g) Gin; (h) Cin; (i) Tn3 resolvase; (j) TndX; (k) XerC; and (l) XerD.

79. (Original) The method of claim 77, wherein the recombination sites which recombine with each other comprise *att* sites having identical seven base pair overlap regions.

80. (Original) The method of claim 79, wherein the first three nucleotides of the seven base pair overlap regions of the recombination sites which recombine with each other comprise nucleotide sequences selected from the group consisting of:

- (a) AAA; (b) AAC; (c) AAG; (d) AAT; (e) ACA; (f) ACC; (g) ACG; (h) ACT; (i) AGA; (j) AGC; (k) AGG; (l) AGT; (m) ATA; (n) ATC; (o) ATG; and (p) ATT.

81. (Original) The method of claim 79, wherein the first three nucleotides of the seven base pair overlap regions of the recombination sites which recombine with each other comprise nucleotide sequences selected from the group consisting of:

(a) CAA; (b) CAC; (c) CAG; (d) CAT; (e) CCA; (f) CCC; (g) CCG; (h) CCT; (i) CGA; (j) CGC; (k) CGG; (l) CGT; (m) CTA; (n) CTC; (o) CTG; and (p) CTT.

82. (Original) The method of claim 79, wherein the first three nucleotides of the seven base pair overlap regions of the recombination sites which recombine with each other comprise nucleotide sequences selected from the group consisting of:

(a) GAA; (b) GAC; (c) GAG; (d) GAT; (e) GCA; (f) GCC; (g) GCG; (h) GCT; (i) GGA; (j) GGC; (k) GGG; (l) GGT; (m) GTA; (n) GTC; (o) GTG; and (p) GTT.

83. (Original) The method of claim 79, wherein the first three nucleotides of the seven base pair overlap regions of the recombination sites which recombine with each other comprise nucleotide sequences selected from the group consisting of:

(a) TAA; (b) TAC; (c) TAG; (d) TAT; (e) TCA; (f) TCC; (g) TCG; (h) TCT; (i) TGA; (j) TGC; (k) TGG; (l) TGT; (m) TTA; (n) TTC; (o) TTG; and (p) TTT.

84. (Original) The method of claim 77, further comprising inserting the nucleic acid segments joined in step (b) into a vector.

85. (Original) The method of claim 77, wherein the joined nucleic acid segments undergo intramolecular recombination to form a circular molecule.

86. (Original) The method of claim 85, wherein the recombination sites which undergo recombination to form the circular molecule are located at the 5' and 3' termini of the one or more of the nucleic acid segments.

87. (Original) The method of claim 77, wherein one or more of the nucleic acid segments encodes a selectable marker.

88. (Original) The method of claim 77, wherein one or more of the nucleic acid segments contains an origin of replication.

89. (Original) The method of claim 77, wherein some or all of the nucleic acid segments comprise nucleic acid molecules of one or more libraries.

90. (Original) The method of claim 77, wherein the one or more libraries comprise polynucleotides which encode variable domains of antibody molecules.

91. (Original) The method of claim 90, wherein at least one of the nucleic acid segments encodes a polypeptide linker for connecting variable domains of antibody molecules.

92. (Original) The method of claim 91, wherein the one or more libraries comprise polynucleotides which encode variable domains of antibody light and heavy chains.

93. (Original) The method of claim 77, further comprising screening to identify nucleic acid molecules which encode proteins having one or more identifiable activities.

94. (Original) The method of claim 93, wherein the one or more identifiable activities comprise binding specificity for one or more antigens.

95. (Original) The method of claim 93, wherein the one or more identifiable activities comprise an enzymatic activity.

96. (Original) The method of claim 93, wherein the one or more identifiable activities comprise an activity associated with a selectable marker.

97. (Original) The method of claim 77, wherein at least two of the nucleic acid segments encode expression products involved in the same biochemical pathway or biological process.

98. (Original) The method of claim 97, wherein the nucleic acid segments encode at least two different subunits of a multimeric enzyme complex.

99. (Original) The method of claim 77, wherein the nucleic acid segments encode at least two different enzymes which participate in reactions in the same biochemical pathway.

100. (Original) The method of claim 77, wherein the biochemical pathway leads to the production of an antibiotic or a carbohydrate.

101. (Canceled)

102. (Canceled)

103. (Original) A method of making a population of recombinant host cells comprising introducing nucleic acid segments joined by the method of claim 77 into a host cell.

104. (Original) A method of altering properties of a cell comprising introducing into the cell nucleic acid segments joined by the method of claim 77.

105. (Original) The method of claim 99, wherein the biochemical pathway leads to the post-translational modification of proteins.

106. (Original) The method of claim 105, wherein the post-translational modification is glycosylation or sialation.

107. (Original) The method of claim 106, wherein the cell is a bacterial cell.

108. (Previously Presented) A method of synthesizing a protein comprising:

- (a) providing a nucleic acid molecule comprising at least one recombination site and comprising a coding sequence containing at least one suppressible stop codon;
- (b) providing a vector comprising at least one recombination site and a coding sequence;
- (c) causing recombination *in vitro* such that the nucleic acid molecule is inserted into the vector to produce a modified vector with the two coding sequences connected in frame and separated by said stop codon;
- (d) transforming a host cell which expresses a suppressor tRNA with the modified vector; and
- (e) causing expression of the two coding sequences such that a fusion protein encoded by at least a portion of both of the coding sequences is produced.

109. (Original) The method according to claim 108, wherein the stop codon is selected from the group consisting of *amber*, *opal* and *ochre* codons.

110. (Original) The method according to claim 108, wherein the vector comprises a gene which encodes at least one suppressor tRNA molecule.

111. (Original) The method according to claim 108, wherein the chromosome of the host cell comprises a gene which encodes at least one suppressor tRNA molecule.

112. (Original) The method according to claim 108, further comprising the steps of transforming the host cell with a nucleic acid molecule comprising a gene which encodes at least one suppressor tRNA molecule.

113. (Original) The method according to claim 108, wherein the fusion protein comprises an N- or C-terminal tag encoded by at least a portion of the vector.

114. (Original) The method according to claim 113, wherein the tag is selected from the group consisting of: (a) glutathione S-transferase; (b) β -glucuronidase; (c) green fluorescent protein; (d) yellow fluorescent protein; (e) red fluorescent protein; (f) cyan fluorescent protein; (g) maltose binding protein; (h) a six histidine tag; and (i) an epitope tag.

115. (Previously Presented) A method for determining the gene expression profile in a cell or tissue comprising:

- (a) generating at least one population of cDNA molecules from RNA obtained from the cell or tissue, wherein the individual cDNA molecules of the population comprise at least one recombination site capable of recombining with at least one recombination site present on cDNA molecules of the same or a different population;
- (b) contacting the nucleic acid molecules of (a) with one or more recombination proteins under conditions which cause the nucleic acid molecules to join;
- (c) determining the sequence of the joined nucleic acid molecules; and
- (d) comparing the sequence of said joined nucleic acid molecules to nucleic acid sequences cataloged in public databases to identify the gene expression profile.

116. (Original) The method of claim 115, wherein the joined cDNA molecules are inserted into a vector which contains sequencing primer binding sites flanking the insertion site.

117. (Original) The method of claim 115, wherein the joined cDNA molecules are separated by *attB* recombination sites.

118. (Original) The method of claim 117, wherein the *attB* recombination sites which recombine with each other have identical seven base pair overlap regions.

119. (Original) The method of claim 115, wherein the joined cDNA molecules contain between about 10 and about 30 nucleotides which corresponds to the RNA obtained from the cell or tissue.

120-142. (Canceled)

143. (Currently Amended) ~~The method of claim 7, wherein said recombination between the one or more recombination sites of the members of said at least first population and the one or more recombination sites of the at least one target nucleic acid molecule occurs *in vitro*.~~

A method of producing a population of hybrid nucleic acid molecules comprising:

- (a) mixing at least a first population of nucleic acid molecules, wherein one or more nucleic acid molecules of said population comprises one or more recombination sites, with at least one target nucleic acid molecule comprising one or more recombination sites;
- (b) causing some or all of the nucleic acid molecules of the at least first population to recombine *in vitro* with all or some of the target nucleic molecules, thereby forming the population of hybrid nucleic acid molecules; and
- (c) selecting for the population of hybrid nucleic acid molecules and against the first population of nucleic acid molecules and against the target nucleic acid molecules.

144. (Currently Amended) ~~The method of claim 60, wherein said recombination between the at least one recombination site of the first population to create a second population occurs *in vitro*.~~

A method of cloning at least one nucleic acid molecule comprising:

- (a) providing a first population of nucleic acid molecules wherein all or a portion of such molecules are flanked by a first and a second recombination site;
- (b) providing at least one nucleic acid segment encoding a polypeptide selected from the group consisting of: (i) the Fc portion of an immunoglobulin; (ii) β -glucuronidase; (iii) a fluorescent protein; (iv) a purification tag; (v) an epitope tag; (vi) maltose binding protein; (vii) a six histidine tag; and (viii) glutathione S-transferase, flanked by a third and a fourth recombination site, wherein either the first or the second recombination site is capable of recombining with either the third or the fourth recombination site;
- (c) conducting a recombination reaction *in vitro* such that all or a portion of the nucleic acid molecules in the population is recombined with the segment to form a second population of nucleic acid molecules; and
- (d) cloning the second population of nucleic acid molecules.

145. (Previously Presented) A method of producing a nucleic acid molecule comprising:

- (a) providing a first nucleic molecule comprising at least one recombination site and comprising a coding sequence containing at least one suppressible stop codon;
- (b) providing a second nucleic acid molecule comprising at least one recombination site and a coding sequence; and
- (c) causing recombination *in vitro* such that the first nucleic acid molecule is joined to the second nucleic acid molecule to produce a third nucleic acid molecule in which the two coding sequences are connected in frame and are separated by said stop codon.

146. (Previously Presented) The method of claim 145, wherein said first nucleic acid molecule and/or said second nucleic acid molecule and/or third nucleic acid molecule is a vector.

147. (Previously Presented) The method of claim 145, further comprising:

- (d) transforming a host cell which expresses a suppressor tRNA with the third nucleic acid molecule.

148. (Previously Presented) The method of claim 145, wherein the stop codon is selected from the group consisting of *amber*, *opal* and *ochre* codons.

149. (Previously Presented) The method of claim 145, wherein said first and/or second nucleic acid molecule comprises a gene which encodes at least one suppressor tRNA molecule.

150. (Previously Presented) The method of claim 147, wherein the chromosome of the host cell comprises a gene which encodes at least one suppressor tRNA molecule.